

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C07H 21/00, C12Q 1/68</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 97/11084</b> <b>(43) International Publication Date:</b> 27 March 1997 (27.03.97)
<b>(21) International Application Number:</b> PCT/US96/13134 <b>(22) International Filing Date:</b> 12 August 1996 (12.08.96) <b>(30) Priority Data:</b> 08/531,132 20 September 1995 (20.09.95) US <b>(71) Applicant:</b> THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 22nd floor, 300 Lakeside Drive, Oakland, CA 94162-3550 (US). <b>(72) Inventors:</b> MATHIES, Richard, A.; University of California, Dept. of Chemistry, 312 Hildebrand, Berkeley, CA 94720 (US). GLAZER, Alexander, N.; University of California, Dept. of Molecular and Cell Biology, 229 Stanley Hall, Berkeley, CA 94720 (US). JU, Jingyue; University of California, Dept. of Chemistry, 329 Hildebrand, Berkeley, CA 94720 (US). <b>(74) Agents:</b> ROWLAND, Bertram, I. et al.; Flehr, Hohbach, Test, Albritton & Herbert, Suite 3400, 4 Embarcadero Center, San Francisco, CA 94111-4187 (US).		<b>(81) Designated States:</b> CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> UNIVERSAL SPACER/ENERGY TRANSFER DYES  <b>(57) Abstract</b> <p>Fluorescent labels are provided employing energy absorber/donor components and energy acceptor/fluorescer components joined together by a spacer which comprises sugar phosphate monomeric units, particularly ribosyl phosphate monomeric units, where the sugar groups are free of active hydroxyl groups. Particularly, an energy transfer component is substituted at the 5' position of the spacer chain, while the other energy transfer component is substituted at the 1' position of the 3' terminal ribosyl group of the label forming an ET cassette for linking to a nucleic acid sequence with any compositions. By employing combinations of ET components, with a common energy absorber/donor and different fluorescers, one can provide for families of labels which can be tagged to any target molecules and which can be excited at a single wavelength and fluoresce at different wavelengths with large Stokes shifts. The compositions find particular application in sequencing.</p>		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France				

## UNIVERSAL SPACER/ENERGY TRANSFER DYES

### INTRODUCTION

#### Technical Field

5       The technical field of this invention is fluorescent labels.

#### Background

There is an increasing demand to be able to identify and quantify components of mixtures. The greater the complexity of the mixture, the greater the interest in being able to simultaneously detect a plurality of the components present. As  
10       illustrative of this situation is DNA sequencing, where it is desirable to efficiently excite from one to four fluorescently tagged components with a laser source at a single wavelength, while providing for fluorescent signal emission at a plurality of distinctive wavelengths. In this situation, the different labels should not adversely  
15       affect the electrophoretic mobility of the sequences to which they are attached.

Currently, there are four methods used for automated DNA sequencing: (1) the DNA fragments are labeled with one fluorophore and then the fragments run in adjacent sequencing lanes (Ansorge et al., *Nucleic Acids Res.* 15:4593-4602 (1987)); (2) the DNA fragments are labeled with four different fluorophores and all the  
20       fragments are electrophoretically separated and detected in a single lane (Smith et al., *Nature* 321:674-679 (1986)); (3) each of the dideoxynucleosides in the termination reaction is labeled with a different fluorophore and the four sets of fragments are run in the same lane (Prober et al., *Science* 238:336-341 (1986)); or (4) the sets of DNA fragments are labeled with two different fluorophores and the

DNA sequences coded with the dye ratios (Huang et al., *Anal. Chem.* 64:2149-2154 (1992)).

All of these techniques have significant deficiencies. Method 1 has the potential problems of lane-to-lane variations in mobility, as well as a low throughput. Methods 2, 3, and 4 require that the dyes be well excited by one laser source and that they have distinctly different emission spectra. In practice, it is very difficult to find two or more dyes that can be efficiently excited with a single laser and that emit well separated fluorescent signals. As one selects dyes with distinctive red-shifted emission spectra, their absorption maxima will also move to the red and all the dyes can no longer be efficiently excited by the same laser source. Also, as more different dyes are selected, it becomes more difficult to select all the dyes such that they cause the same mobility shift of the labeled molecules.

It is therefore of substantial interest that improved methods be provided which allow for multiplexing of samples, so that a plurality of components can be determined in the same system and in a single run. It is also desirable for each label to have strong absorption at a common wavelength, to have a high quantum yield for fluorescence, to have a large Stokes shift of the emission, that the various emissions be distinctive, and that the labels introduce the same mobility shift. It is difficult to accomplish these conflicting goals by simply labeling the molecules with a single dye.

#### Relevant literature

Ju et al., *Proc. Natl. Acad. Sci. USA* 92:4347-4351 (1995), and Ju et al., *Anal. Biochem.*, in press (1995) describe fluorescent energy-transfer (ET) primers for DNA sequencing. Also of interest is Wang et al., *Anal. Chem.* 67:1197-1203 (1995) which describes the use of ET primers for PCR product labeling and detection.

#### SUMMARY OF THE INVENTION

Fluorescent label compositions are provided comprising a combination of an energy absorber/donor component and an energy acceptor/fluorescer component bonded to a spacer comprising sugar phosphate links free of purine and pyrimidine

bases forming an ET cassette. By providing a plurality of fluorescent dye compositions, having the same absorber/donor and different energy acceptor/fluorescer components, a family of fluorescent dye compositions (ET cassette) are provided which can label target molecules with any sequences and, which can be excited at the same wavelength, emit at widely spaced wavelengths, and provide for the same effect on mobility in electrophoresis, when associated with different nucleic acids. Also, the compositions can be readily synthesized using conventional polynucleotide synthetic chemistries.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the comparison of the fluorescence emission intensity of four energy transfer (ET) primers.

Figure 2 shows a portion of 4-color analyzed data (nucleotides 10 to 230) of DNA sequencing profile of M13mp18 DNA using the ET primer FS<sub>6</sub>F, FS<sub>6</sub>J, FS<sub>6</sub>T, FS<sub>6</sub>R and Sequenase 2.0. Primer concentration: 0.4 pmol; DNA template: 1.6 µg (0.4 µg for each base extension). The CE sequencing protocols were similar as described (Ju et al., *Anal. Biochem.*, in press (1995)).

#### DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Novel fluorescent compositions, kits comprising the compositions, and methods of use are provided. The compositions comprise an energy absorber/donor component and an energy acceptor/fluorescer component joined together by a spacer comprising sugar, e.g., ribosyl phosphate links, where the hydroxyl at the 1'-position is substituted by hydrogen or etherified. The fluorescent compounds are useful as labels, particularly in association with nucleic acids. By employing a plurality of the compounds, where the compounds share a common energy absorber/donor component and common spacers, but vary as to the energy acceptor/fluorescer component, one can provide for a family of fluorescent labels which can be excited at the same wavelength and provide for widely spaced emission wavelengths. The family of compounds find particular use for labeling nucleic acids to be separated by electrophoresis, particularly exemplified by nucleic acid sequencing.

The subject labels will be characterized by having an energy absorber/donor component bonded to a spacer having at least three sugar-phosphate units and an energy acceptor/fluorescer component bonded to the spacer separated from the donor component by at least three sugar-phosphate units. The spacer with the donor and acceptor attached forms an ET cassette which can be used to tag any target molecules. One terminus of the label will have a functionality for reacting with a phosphate or hydroxyl, particularly of a nucleoside or nucleotide, normally the only functionality available for reacting. Generally, the number of sugar-phosphate moieties will be not greater than 15, usually not greater than 12, preferably not greater than about 9, and at least 3, preferably at least about 4, more usually in the range of about 5-8. While various sugars may be employed of from 5-6 carbon atoms, conveniently the sugar employed is ribose. Two of the hydroxyl groups of the sugar will be involved in the phosphate ester linkages while the remaining hydroxyl groups, except for the hydroxyl group at 1'-position, will either be substituted, particularly etherified by alkyl of from 1-3 carbon atoms, so as to be chemically inert, or replaced with hydrogen. The 1'-position may serve as a site for linking to an energy transfer component or will be modified so as to be unreactive as described above. Particularly of interest as the sugar used in the spacer will be 1', 2'-dideoxy ribose, where the 3' and 5' positions will serve as phosphate ester sites in the chain. If desired, the terminal 5' position may be used as the site for linking to one of the energy transfer components.

The energy transfer ("ET") components may be at any sites of the spacer which allow for efficient energy transfer. Therefore, the energy transfer components will generally be spaced apart for about 3-9, more usually for about 4-8 sugar phosphate moieties. The particular spacing is not critical to this invention, so long as a similar spacing is used for each compound to be used in combination. Conveniently, the 5' terminal hydroxyl group may be the site for one of the energy transfer components, particularly the donor energy transfer component. Also, the other component will be conveniently substituted at a 1'-position, either directly or through a linking group of not more than about 20 atoms, more usually not more than about 12 atoms, where a cyclic intervening group is present, the shorter number of atoms around the ring being counted.

The linking group can be varied widely and is not critical to this invention. The linking groups may be aliphatic, alicyclic, aromatic or heterocyclic, or combinations thereof. Functionalities or heteroatoms which may be present in the linking group include oxygen, nitrogen, sulfur, or the like, where the heteroatom  
5 functionality which may be present is oxy, oxo, thio, thiono, amino, amido, and the like. Any of a variety of linking groups may be employed which do not interfere with the energy transfer and gel electrophoresis, which may include purines or pyrimidines, particularly uridine, thymidine, cytosine, where substitution will be at an annular member, particularly carbon, or on a side chain, e.g. methyl in  
10 thymidine. The ET component may be bonded directly to the base or through a linking group of from 1 to 6, more usually from 1 to 3 atoms, particularly carbon atoms. The linking group may be saturated or unsaturated, usually having not more than about one site of aliphatic unsaturation.

For the ET component linked to the 1'-position, one may advantageously use  
15 the base of a nucleotide as the site for linking the ET component, the 1'-hydroxyl of the sugar, or one may introduce a functionality at the 1'-position, such as amino, carboxyl, and the like. The functionality of the 1'-position may be extended, particularly with a substituted alkylene group of from 2-8, usually 2-6 carbon atoms where the final functionality will depend upon the available functionality present on  
20 the ET component. Usually, the functionality for linking to the ET component will be carboxyl or amino.

Linkages which may be involved include amino (for example, by reductive amination), amide, ethers, either oxy or thio, esters, or the like. The fluorescent label comprising the ET components may be bonded to the nucleic acid molecule by  
25 any convenient linkage, usually a phosphate ester linkage. The subject fluorescent label molecules will normally be at the 5' terminus of the nucleic acid molecule, but in certain situations may be at a different site if desired. For use in sequencing, the subject compositions will normally be at the 5' end of the sequence.

The compositions of interest will, for the most part, have the following formula



Wherein:

D is the donor component;

A is the acceptor component;

10 S is the spacer monomer;

S' is a modified spacer monomer, where the modification is at the 1'-position for linkage to A;

X is the target molecule to be labeled, which can be polynucleotides, polypeptides or the like. X contains an activated phosphate group for reaction with  
 15 an hydroxyl, protected hydroxyl, or a mono- or polynucleotide particularly a deoxyribonucleotide, generally of from 1-30, more usually of from 1-20 nucleotides; and

n is in the range of 2-14, more usually in the range of 4-7.

The energy donors will generally be compounds which absorb in the range of  
 20 about 300-800 nm more usually in the range of about 450-700 nm, and are capable of transferring energy to a fluorescer, which fluorescer generally absorbs light of a wavelength 15 nm, more usually 20 nm or higher, than the absorption wavelength of the donor. The fluorescers will generally emit in the range of about 400-900 nm. A wide variety of energy absorbers may be employed, such as fluorescein, BODIPY,  
 25 DAPI, Indo-1, coumarin, dansyl, cyanine dyes, or the like. Energy acceptors/fluorescers may include some of the compounds indicated above, which fluoresce at or above about 450 nm, as well as compounds such as rhodamines, fura-2, and diverse cyanine dyes. For the most part, the ET components will be non-intercalating, stable under the conditions of electrophoresis, and have high extinction  
 30 coefficients and high fluorescence efficiency.

The energy donor will have a strong molar absorbance coefficient at the desired excitation wavelength, desirably greater than about  $10^4$ , preferably greater



than about  $10^5 \text{ cm}^{-1}\text{M}^{-1}$ . The excitation maximum of the donor and the emission maximum of the acceptor will be separated by at least about 15 nm or greater. The spectral overlap integral between the emission spectrum of the donor and the absorption spectrum of the acceptor chromophore and the distance between the chromophores will be such that the efficiency of energy transfer from donor to acceptor will typically range from about 20 to 100%. For the most part, the donor-acceptor dyes will have molecular weights of less than about 2kD, generally less than about 1.5kD.

While for the most part, single pairs of ET components will be used, there can be situations where up to three different chromophores will be utilized in sets, usually differing by not more than two different chromophores. That is, normally the energy donor is the same and the final fluorescer and one other intermediate chromophore will be different. By using more ET components, one may greatly extend the Stokes shift, so that one may excite in the visible wavelength range and have emission in the infra-red wavelength range, usually below about 1,000 nm, more usually below about 900 nm. Detecting light in the infra-red wavelength range has many advantages, since it will not be subject to interference from Raman and Rayleigh light resulting from the excitation light and the background emission signals from biological samples are lower in the red. In order to maintain the mobility constant, one may use the same number of ET components on each of the labels, having two to three of the same ET components on each of the labels.

Where the subject labels are used as primers, they can have nucleotide sequences of from about 8-30, more usually from about 8-20 nucleotides, where the sequence is complementary to the desired priming site or priming site in various commercially available vectors. The vectors may include single-stranded filamentous bacteriophage vectors, the bacteriophage lambda vector, Charon vectors, pUC vectors, pGEM vectors, or the like. Thus, the subject labels would be extended from the common sequence of the vector into the cloned sequence. For example, universal primers may be prepared, where the primer may be any one of the universal primers, having been modified by bonding of the label to the primer. Thus, various commercial primers are available, such as primers from pUC/M13,  $\lambda$ gt10,  $\lambda$ gt11, and the like. See, Sambrook et al., *Molecular Cloning: A Laboratory*

*Manual*, 2nd ed., CSHL, 1989, Section 13.

The subject invention finds particular application with nucleic acid chains, where the nucleic acid chains find use as primers in sequencing, the polymerase chain reaction, particularly for sizing, or other systems where primers are employed for nucleic acid extension and one wishes to distinguish between various components of a mixture as related to the particular labels. For example, in sequencing, universal primers may be employed, where a different pair of ET components is used for each of the different dideoxynucleotides used for the extension during the sequencing reaction. DNA sequences are cloned in an appropriate vector having a primer sequence joined to the sequence to be determined. Different 2', 3'-ddNTPs are employed, so that termination occurs at different sites, depending upon the particular ddNTP which is present in the chain extension. By employing the subject primers, each ddNTP will be associated with a particular label. After extension with the Klenow fragment, the resulting fragments may then be separated in a single lane by electrophoresis or in a single capillary by electrophoresis, where one can detect the terminating nucleotide by virtue of the fluorescence of the label.

The subject compounds may have an active phosphate for linking to a nucleotide. Various active phosphates include phosphoamidites, where the oxygen is substituted with an alkyl group of from 1-3 carbon atoms, cyanoalkyl, particularly of from 2-3 carbon atoms, etc. The amino group will generally be dialkylated where the alkyl groups will be from 1-4, more usually 2-3 carbon atoms. Alternatively, phosphite esters may be employed, where the available oxygens will be substituted with alkyl groups of from 1-3 carbon atoms.

The subject compounds may be readily synthesized in accordance with conventional techniques, where the synthesis follows conventional synthetic procedures for nucleotides. Thus, one can readily oligomerize 1'-Y 3'-activated, 5'-protected 2'-deoxyribose in the same manner as DNA is synthesized, where Y is hydrogen, a functional group, or an ET component joined directly or through a linking group.

Kits are provided having combinations of labels, usually at least 2. Each of the labels will have the donor-acceptor pair, usually with the same backbones, where the labels will be separated along the backbone and give comparable mobility in the

separation method to be used. Each of the labels in a group to be used together will absorb at about the same wavelength and emit at different wavelengths. Each of the labels in the group will have about the same effect on mobility in the separation method.

- 5        The kits will generally have up to about 6, usually about up to about 4 different labels which are matching, but may have 2 or more sets of matching labels, having 2-6 different labels.

The following examples are offered by way of illustration and not by way of limitation.

10

### EXPERIMENTAL

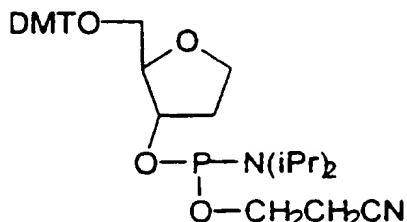
#### **Design and Synthesis of Energy Transfer Fluorescent Dye Tagged Oligonucleotide Labels with a Universal Spacer**

- The M13 (-21) universal primer containing 18-nucleotides with the following  
15    sequence



- were synthesized with donor-acceptor fluorophore pairs separated by six 1', 2'-  
20    dideoxyribose phosphates (S) which were introduced using 5'-dimethoxytrityl-1', 2'-dideoxyribose-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (dSpacer CE Phosphoramidite, Glen Research) (Structure 1) on a DNA synthesizer

25

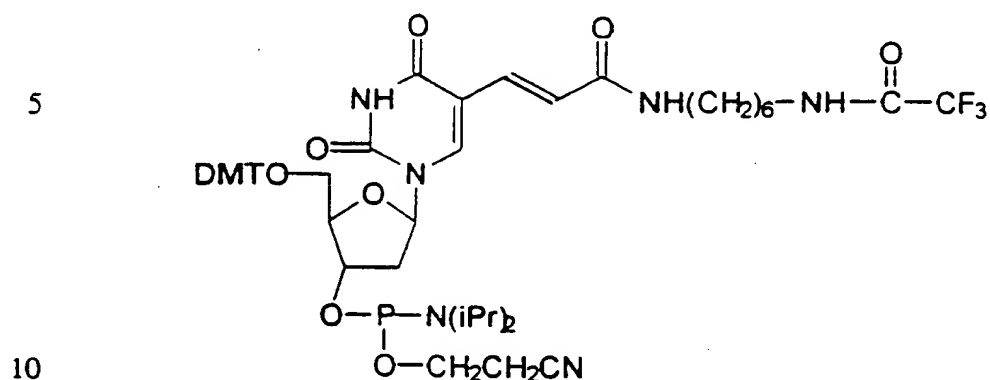


30

#### **Structure 1. dSpacer CE Phosphoramidite**

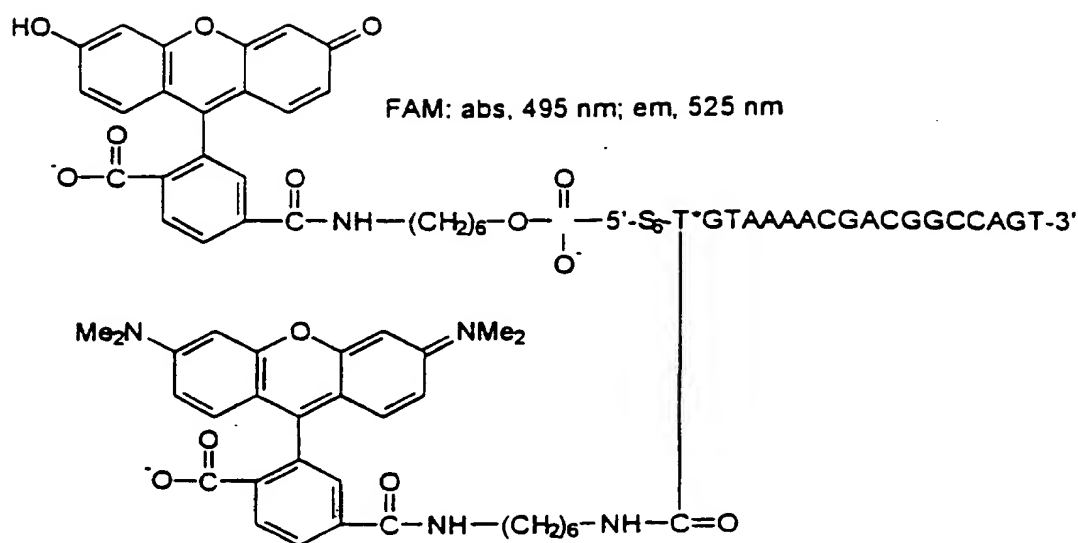
The 18-mer contains a modified base T\* introduced by the use of 5'-dimethoxytrityl-5-[N-(trifluoroacetylaminohexyl)-3-acrylimido]-2'-deoxyUridine, 3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (Amino-Modifier C6 dT, Glen Research)

(Structure 2),



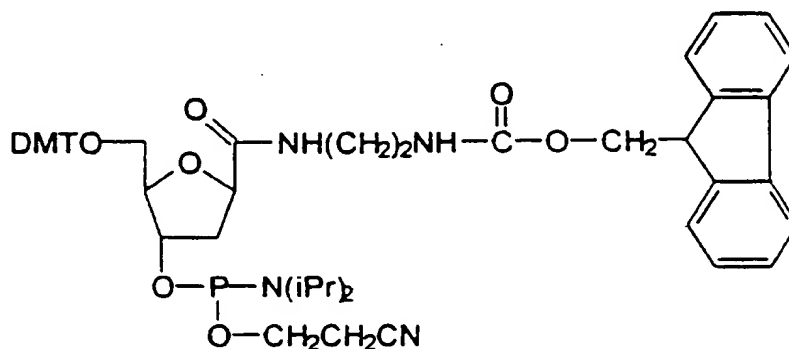
Structure 2. Amino-Modifier C6 dT

which has a protected primary amine linker arm at the C-5 position. The T\* was attached to the 5' end of the nucleotide sequences before the spacer. The donor dye was attached to the end of the spacer on the 5' side of the oligomer, and the acceptor dye was attached to the primary amine group on the T\*. The primers are denoted as D-S<sub>6</sub>-A (ET cassette), where D is the donor, A is the acceptor and S<sub>6</sub> indicates the number of spacing sugars between D and A. In all the primers prepared, D = FAM(F), a fluorescein derivative; A = FAM(F), JOE(J), TAMRA(T) or ROX(R) with the last two being rhodamine derivatives. We have also found that carboxyrhodamine-6G is a good substitute of JOE (FAM: 6-carboxyfluorescein; JOE; 2' 7'-dimethoxy-4', 5'-dichloro-6-carboxyfluorescein, TAMRA: N, N, N', N'-tetramethyl-6-carboxyrhodamine; ROX: 6-carboxy-X-rhodamine). As a representative example, the structure of FS<sub>6</sub>T is shown below (Structure 3).

Structure 3. FS<sub>6</sub>T

The advantages of the energy transfer approach described here are (1) that primers with any sequences can be labeled with ET coupled dyes simply by

5 introducing a T\* using Amino modifier dT or other phosphoramidite such as Amine-VN Phosphoramidite (CLONTECH) (Structure 4) at the 5' end of the oligonucleotide sequences, and then six 1', 2'-dideoxyribose phosphates using dSpacer. Donor and fluorescer dyes can be easily attached to this scaffold



Structure 4. Amine-VN Phosphoramidite

forming an ET cassette; (2) a large Stokes shift and much stronger fluorescence

signals can be generated when exciting at 488 nm and (3) no mobility shift adjustment on the 4-color data of the DNA sequencing fragments extended from the  $FS_6(F,J,T,R)$  primers are needed to call sequences.

The absorption and emission spectra of the ET primers are presented in Figure 1. Each ET primer exhibits the characteristic absorption of FAM at 496 nm as well as strong absorption at 525 nm due to JOE in  $FS_6J$ , at 555 nm due to TAMRA in  $FS_6T$  and at 585 nm due to ROX in  $FS_6R$ . The fluorescence emission spectra of the ET primers are dominated by the acceptor emissions. While the emission maximum of  $FS_6F$  is at 525 nm, the emission of  $FS_6J$  with 488-nm excitation is Stokes-shifted to 555 nm, that of  $FS_6T$  is shifted to 580 nm, and that of  $FS_6R$  is shifted to 605 nm. In the case of  $FS_6R$ , the Stokes shift is over 100 nm. Figure 1 also presents emission spectra of the single dye-labeled primers measured at the same molar concentration as that of the corresponding ET primers. Substantial enhancement of the ET primer emission intensity is observed compared to the corresponding single dye-labeled primers, indicating that efficient energy transfer is occurring. The fluorescence intensity improvements derived from Figure 1 are:  $FS_6F = 1.7 \times FAM$ ;  $FS_6J = 3 \times JOE$ ;  $FS_6T = 10 \times TAMRA$ ;  $FS_6R = 12 \times ROX$ .

#### DNA Sequencing with ET ( $FS_6F$ , $FS_6J$ , $FS_6T$ , $FS_6R$ ) Primers.

The successful application of the ET dye-labeled primers using the universal spacer for DNA sequencing was also realized. The primers were shown to be good substrates for the DNA polymerase without any anomalies. Figure 2 shows a portion of 4-color DNA sequencing data (10-230 nucleotides) using the  $FS_6N$  primer set. The reaction was performed using equal amounts of primer (0.4 pmol) and 0.4  $\mu$ g of template DNA for each base extension. The 4-color CE sequencing profile was obtained after simply applying a matrix transformation on the raw data to correct for the crosstalk between the four channels. The sequence can be called without applying any mobility shift adjustment. The analysis of these data using more sophisticated peak calling software should lead to even better base calling and longer reads.

It is evident that with the universal spacer (ET cassette) method described above, we are able to synthesize broad families of dye-labeled biopolymers or other

target molecules having a common donor and 2, 3, 4 or more different acceptors. With this approach, all the oligonucleotide primers and probes for sequencing, PCR and hybridization etc. can be tagged with ET coupled dyes. Such classes of donor-acceptor labeled molecules find wide application in multiple-color genetic analyses.

5

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

10

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

WHAT IS CLAIMED IS:

1. A compound comprising an energy donor component and an acceptor component absorbing energy transferred by said energy donor component, said  
5 components linked by a backbone comprising sugar phosphate ester monomer linkages with the 1-hydroxyl group being replaced by a hydrogen or etherified.
2. A compound according to Claim 1, wherein said backbone comprises from 3 to  
15 1, 2-dideoxyribosyl 3, 5-phosphate ester monomer linkages.
- 10 3. A compound according to Claim 2, wherein said energy donor component is covalently bonded at the 5 backbone terminus and said acceptor is covalently bonded to the backbone 3-terminal of 1, 2-dideoxyribosyl 3, 5-phosphate ester at the 1-position through a linking group.
- 15 4. A compound according to Claim 3, wherein the 3-terminus of said backbone is an hydroxyl, protected hydroxyl, phosphoramidite, phosphite, nucleotide or oligonucleotide.
- 20 5. A compound of the formula 5- fluorescein substituted poly-1, 2-dideoxyribose-3, 5-phosphate, having from 3 to 15 1, 2-dideoxyribose-3, 5-phosphate monomeric units, and substituted through a linking group at the 1-position of the 3-terminal 1, 2-dideoxyribose-3, 5-phosphate monomeric unit with a fluorescein or a rhodamine.
- 25 6. A compound according to Claim 5, wherein the 3-terminus of said backbone is an hydroxyl, protected hydroxyl, phosphoramidite, phosphite, nucleotide or oligonucleotide.
7. A compound according to Claim 5, wherein said linking group is a carboxyl or  
30 uridyl group.
8. In a method for identifying different nucleic acid sequences using a



combination of fluorescent labels in an electrophoresis separation, said fluorescent labels fluorescing at different wavelengths and each different label joined to a different nucleic acid sequence, the improvement which comprises:

using any of the compounds of the Claims 1 to 7 as said labels.

5

9. A kit comprising at least two compounds according to Claims 1 to 7 differing in said acceptor and having the same energy donor.

1/2

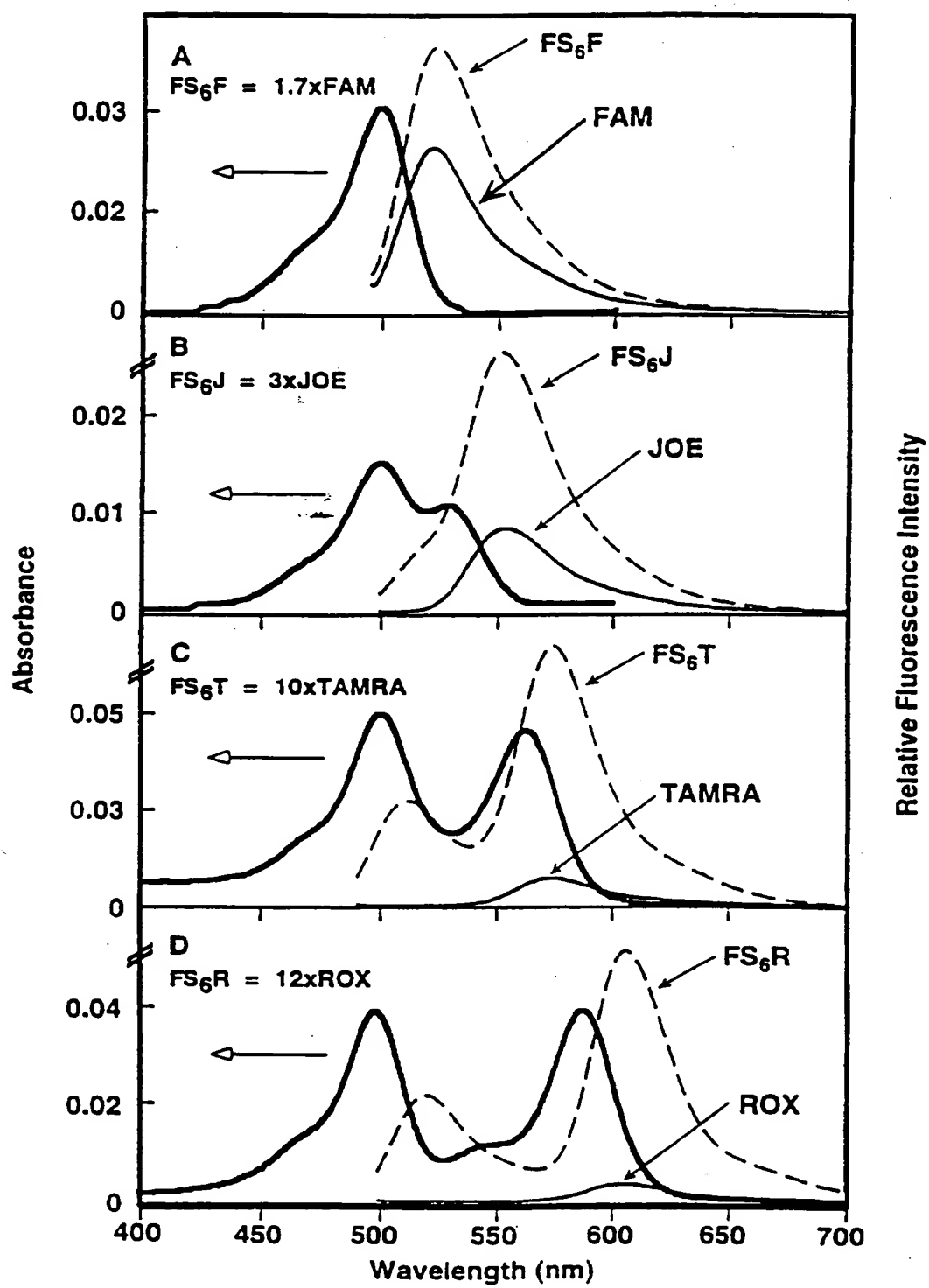


FIG.1

2/2

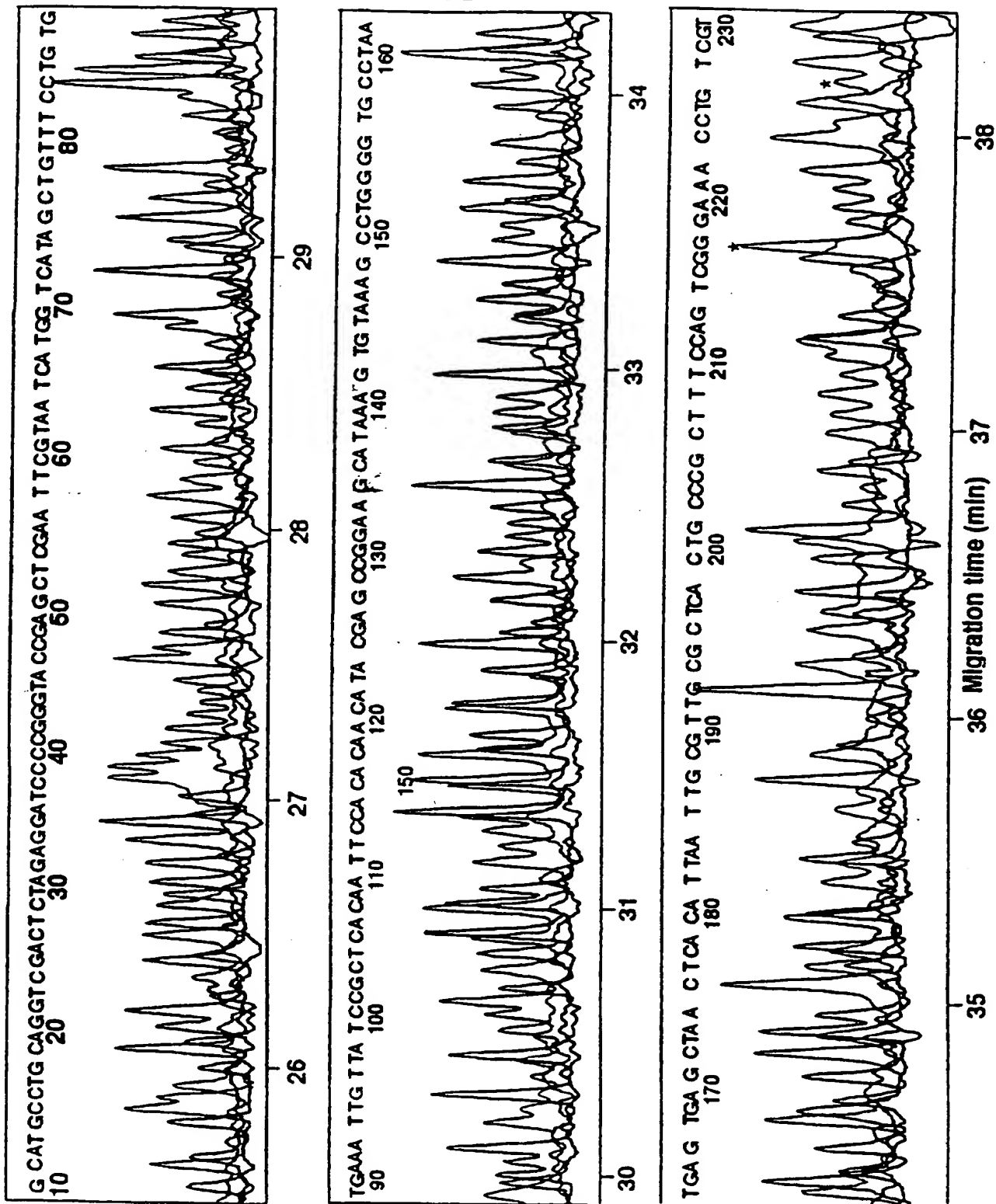


FIG.2

## INTERNATIONAL SEARCH REPORT

International Application No.

PL, US 96/13134

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 C07H21/00 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 C07H C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 21266 A (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 10 August 1995 see abstract	1
A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 92, no. 10, 9 May 1995, WASHINGTON US, pages 4347-4351, XP002019047 I. JU ET AL.: "Fluorescence Energy Transfer Dye-Labeled Primers for DNA Sequencing and Analysis." cited in the application see the whole document	1
A	WO 94 17397 A (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 4 August 1994 see abstract	1

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

\*&\* document member of the same patent family

Date of the actual completion of the international search

21 November 1996

Date of mailing of the international search report

10-12-1996

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax (+31-70) 340-3016

Authorized officer

Scott, J

# INTERNATIONAL SEARCH REPORT

International Application No.

PC, US 96/13134

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DE 41 19 075 A (MAX-PLANCK GESELLSCHAFT ZUR FORDERUNG DER WISSENSCHAFTEN) 17 December 1992 see abstract	1
E	EP 0 731 178 A (HAMAMATSU PHOTONICS K.K.) 11 September 1996 see abstract	1

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PC /US 96/13134

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9521266	10-08-95	AU-A- 1736795 CA-A- 2182516	21-08-95 10-08-95
-----	-----	-----	-----
WO-A-9417397	04-08-94	US-A- 5401847 AU-A- 5993494 JP-T- 8505776	28-03-95 15-08-94 25-06-96
-----	-----	-----	-----
DE-A-4119075	17-12-92	NONE	
-----	-----	-----	-----
EP-A-731178	11-09-96	NONE	
-----	-----	-----	-----